

Evaluating Contamination Risk When Aspirating a Prosthetic Joint Using Microbial Next-Generation DNA Sequencing: An In Vitro Study

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Question: Does NGS Technique “Over Detect”

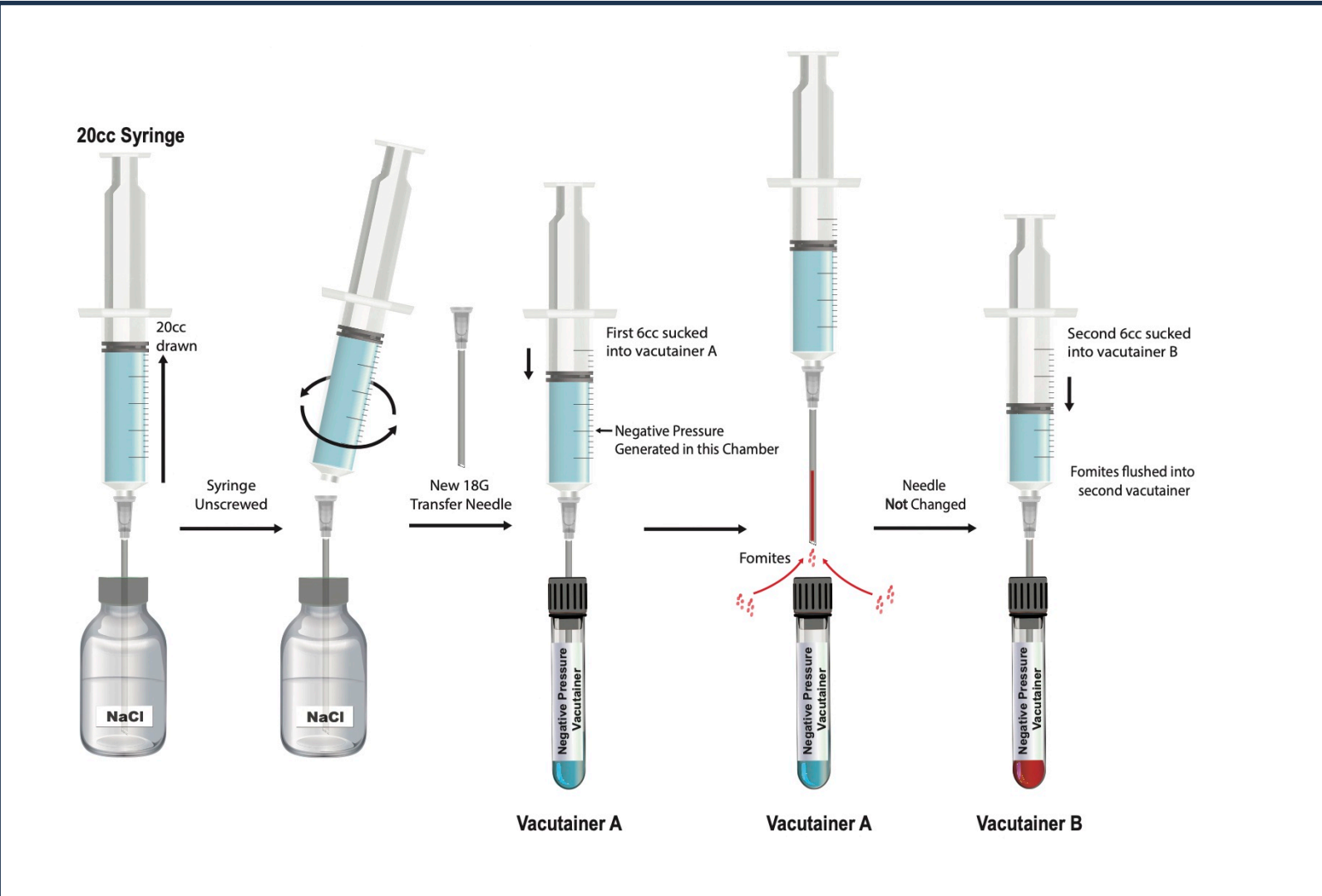
Introduction:

- Up to 50% of cases of documented PJI in recent reports are culture negative¹.
- Molecular methods were developed to improve microbial detection, one common technique being next-generation sequencing (NGS).
- NGS is a highly sensitive detection method, but its specificity is questioned because of discordance with cultures.
- Some NGS false-positive rates are attributed to processing error^{2,3}. However, ...
- Employing rigorous lab quality assurance (QA) protocols, false positive results may instead be a result of **incorrect collection technique** that introduces errant microbiota.
- We hypothesize certain aspiration techniques may contaminate the collection specimen at a level detectable by NGS, but correct technique will have a low false positive rate

Methods:

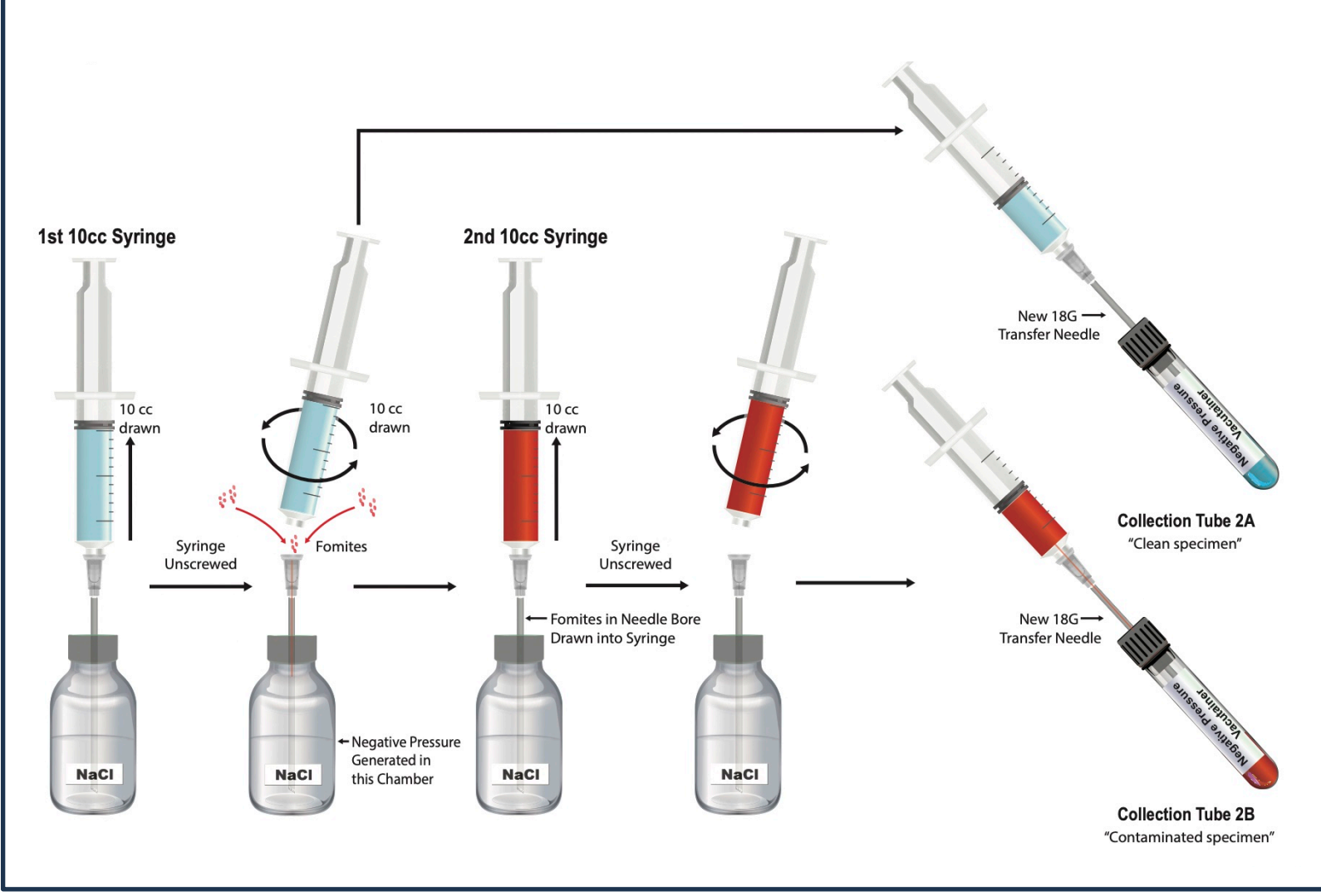
- The “study joint” consisted of a **sterile** 50 mL saline bottle.
- Aspirations were performed under simulated clinical conditions using the same sterile technique, personnel, and procedure room used for patients..
- 20 simulated aspirations performed for each technique.
- Three clinical aspiration techniques were tested (Figure A-C) along with one ambient air contamination technique (Figure D).
- Eighty unopened sterile saline bottles were sent directly for NGS lab testing as the negative control group.
- NGS technique tested for bacterial 16S V1-V2 and fungal ITS targets.

A: Transfer Needle Contamination



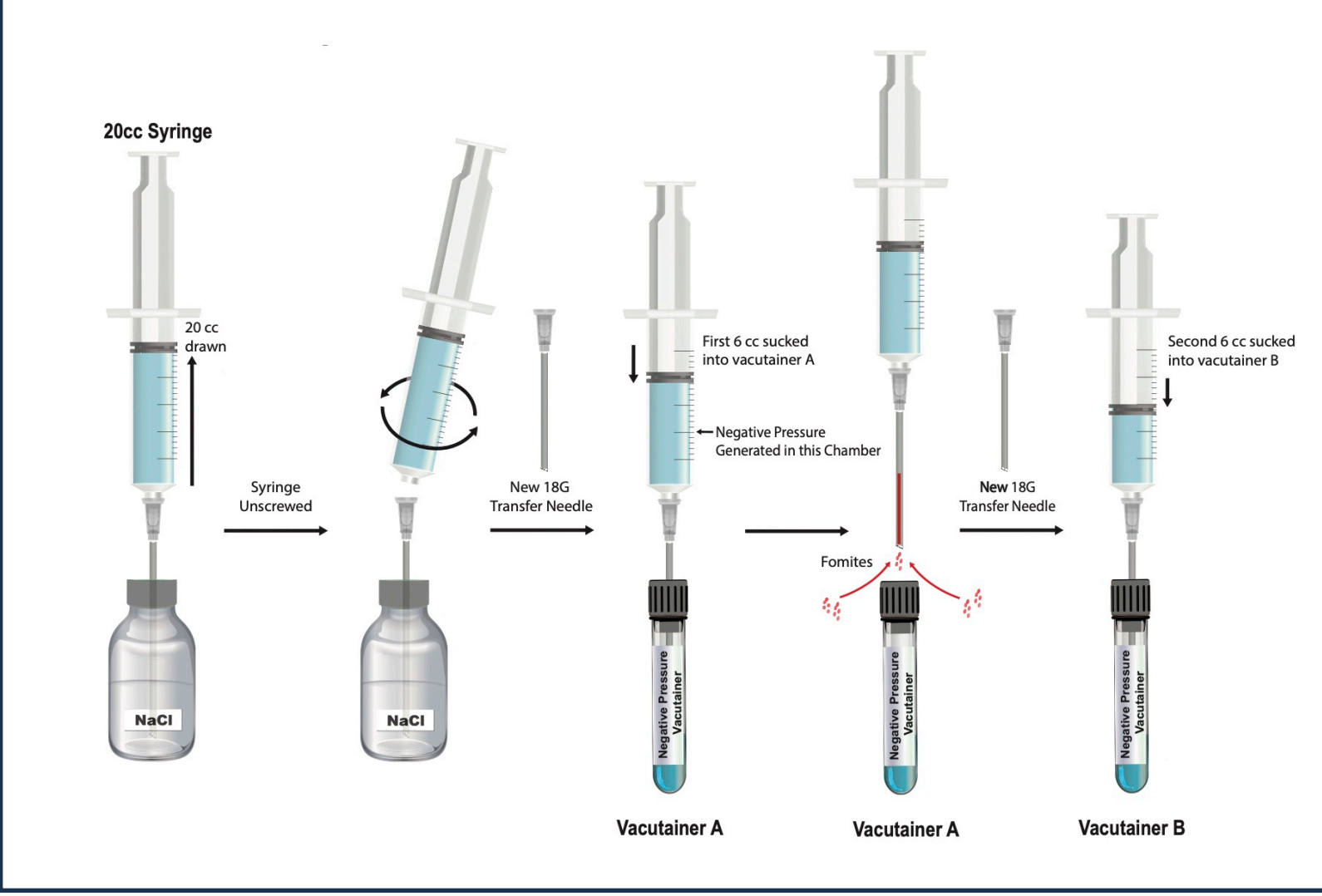
A. Technique 1: Transfer needle contamination. When the transfer needle is removed from the first vacutainer, a **negative-pressure** effect sucks a small amount of ambient air into the needle bore. Because the needle is not changed, the **needle bore contents** are flushed into the second vacutainer.

B: Spinal Needle Contamination



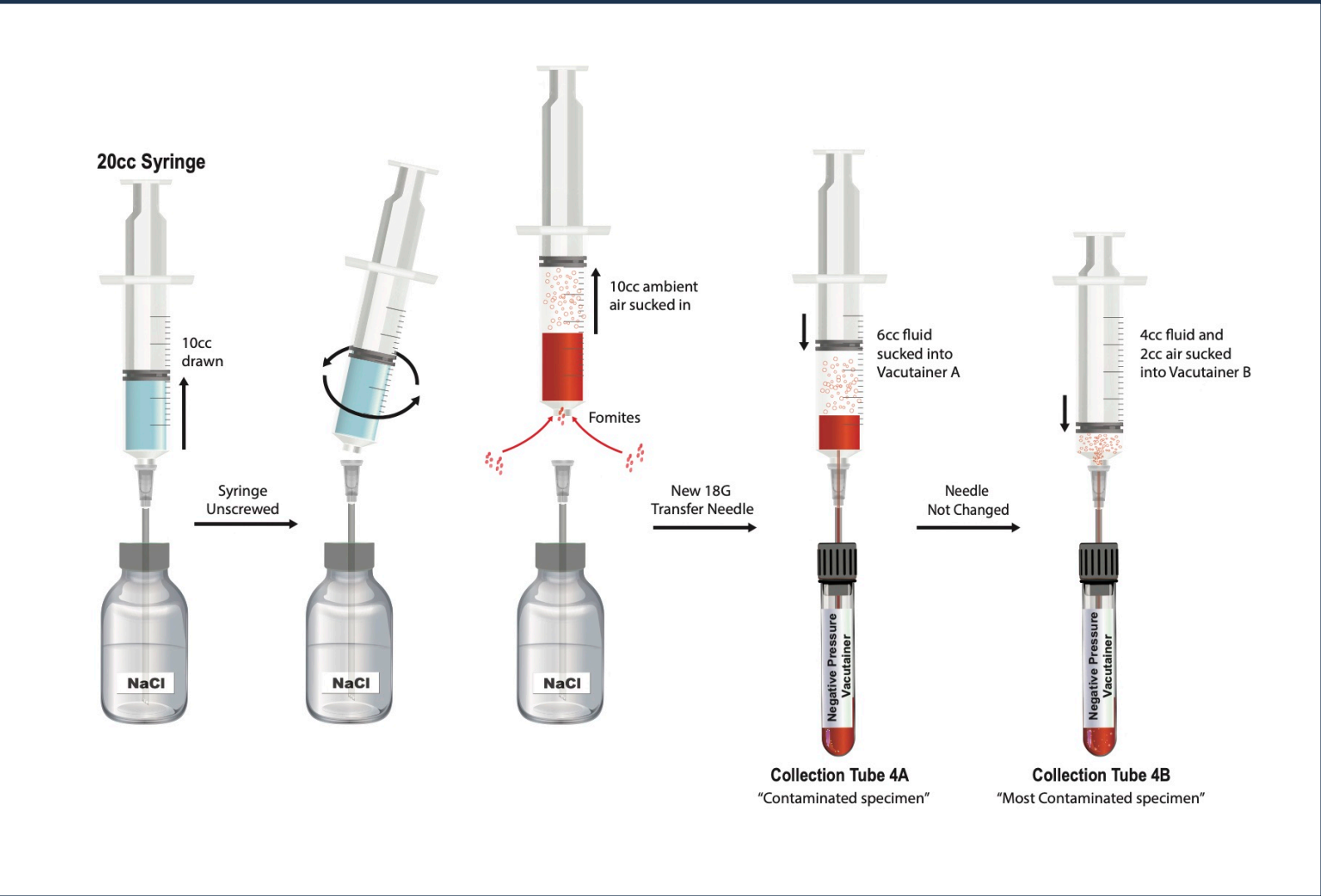
B. Technique 2: Spinal needle contamination. When the 10-mL syringe is detached from the spinal needle, a **negative-pressure** effect sucks a small amount of ambient air into the spinal needle bore. As the second 10-mL syringe withdraws another 10 mL of fluid, the **needle bore contents** are flushed into the syringe, which is transferred into the second vacutainer.

C: Cleanest Technique



C. Technique 3: Cleanest technique. When the transfer needle is removed from the first vacutainer, a negative-pressure effect sucks a small amount of ambient air into the needle bore. This needle is exchanged with a **new needle**. Because all 20 mL of the fluid is removed in one draw, there is **no risk** from spinal needle bore contamination.

D: Amplified Air Technique



D. Technique 4: Air amplified technique. After aspirating 10 mL of fluid with a 20-mL syringe, **10 mL of ambient air** is aspirated into the syringe. The first vacutainer contains 6 mL of fluid. The second vacutainer is filled with 4 mL of saline and **2 mL of aspirated ambient air**. Theoretically, this vacutainer should have the highest rate of NGS detection.

Results: (Table 1)

- For clinical techniques 1-3, only one out of 120 NGS tests was positive (0.8%).
- In the ambient air samples, two out of 20 were positive (10%).
- Of 80 unopened negative control bottles that were processed directly at the testing lab, there was one positive NGS test (1.2%).

Table 1. Results of Next-Generation Sequencing Testing of Aspiration Specimens

Technique Contamination Method	Technique #1 Transfer Needle		Technique #2 Aspiration Needle		Technique #3 Cleanest		Technique #4 Air Amplified	
Vial	1A	1B	2A	2B	3A	3B	4A	4B
Positive NGS samples	0/20	0/20	0/20	1/20	0/20	0/20	0/20	2/20
% positive NGS samples	0%	0%	0%	5%	0%	0%	0%	10%

A = first sample collected; B = second sample collected. **Bold** indicates potential needle bore contamination points.

- Table 2 identifies the microbes detected by NGS.
- In the one positive clinical aspiration, and the 2 air amplified aspirations, the identified microbes are consistent with organisms disseminated via airborne fomites^{3,4}.
- In the negative control bottle, the organism *Bartonella coopersplainsensis*, is considered a cross-contaminant from reagent fluid. Bartonella is a known contaminate of reagent fluid
- Species with read counts below 300 with QA processing, would be excluded from clinical reporting

Table 2. Results (Raw Data) of Next-Generation Sequencing Testing of Aspiration Specimens

Technique	Sample	Specimen	Name of organism(s)	No. of reads
Technique 2	Vacutainer 2B	1	<i>Escherichia coli</i>	22,977
			<i>Enterococcus faecalis</i>	843
			13 organisms < 300 reads	1,646
			Total reads	25,466
Technique 4	Vacutainer 4B	1	<i>Cutibacterium acnes</i>	1,321
			<i>Staphylococcus epidermidis</i>	355
			17 organisms < 300 reads	783
			Total reads	2,459
		2	<i>Naganishia friedmannii</i>	5,725
			Total reads	5,725
Negative control bottle	Bottle 5	1	<i>Bartonella coopersplainsensis</i>	1,177
			13 organisms < 300 reads	743
			Total reads	1,920

Bold indicates total reads from each specimen.

Discussion:

- The 0.8% false-positive may suggest the negative pressure effect draws **airborne fomites** into the needle bore, which contaminated test fluid (Figure 1B).
- The air aspirated samples were not shaken, so second vacutainer is theoretically at higher risk from airborne contamination.
- The 1.2% positivity in the negative control, is explained by the manual “non-routine” extraction from saline bottles, allowing for cross contamination.
- Overall however, NGS has a **low false-positivity rate**.
- Needles exposed to negative-pressure effect should be discarded before fluid transfer.
- Larger test numbers are needed to prove statistical difference.

Key Takeaways:

- Aspiration technique does matter!**
- Always change the needle when transferring fluid.**
- Avoid aspirating air into the needle bore.**
- NGS has a negligible false positive rate with proper technique.**

References:

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